

PRELIMINARY AMENDMENT

NIT-133-06

IN THE CLAIMS

1-29. Canceled.

30. (Currently Amended) An apparatus for recovering a target polynucleotide in a cell comprising:

a substrate being disposed in a separation cell, wherein ~~thea~~ sample solution containing cells each containing polynucleotides and protein is supplied on a surface of the substrate, wherein the substrate has a plurality of independent areas ~~are-formed on theits surface-of-the substrate~~ and each of a single-stranded oligonucleotide probes each having a specific base sequence is immobilized to each of the plurality of independent areas;

capturing means for capturing each of the cells one by one separately on each of the plurality of independent areas;

means for applying a DC field onto a surface of one area of the plurality of independent areas;

temperature measuring means for measuring a temperature of the surface of the substrate at said one area ~~of the areas~~;

heating or cooling means for heating or cooling the surface of the substrate at ~~thesaid one area-of-the-areas~~;

controlling means for controlling selectively the temperature of the surface of the substrate at ~~thesaid one area~~ on the basis of a temperature information obtained by the

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temperature measuring means, by controlling the heating or cooling means,

wherein the controlling means controls the heating or cooling means so as to heat the surface of the substrate at ~~thesaid~~ one area of the areas to a first predetermined temperature to destroy the cell captured at ~~thesaid~~ one area, to liberate the polynucleotides and the proteins from the cell captured at ~~thesaid~~ one area, and to denature the polynucleotides liberated from the cell so as to obtain single-stranded polynucleotides, and the controlling means controls the heating or cooling means so as to cool a solution which contains no polynucleotide and has a pH value of 4 or lower and with which the sample solution on the substrate is replaced, to a second predetermined temperature to form hybrids between the single-stranded polynucleotides and the single-stranded oligonucleotide probes, so as to capture~~ing~~ single-stranded target polynucleotides;

wherein, after separating the single-stranded polynucleotides and the proteins, whereby the hybrids remain on ~~thesaid~~ one area, by electrophoresis under the DC field applied onto the surface of ~~thesaid~~ one area, based on a charge difference between the single-stranded target polynucleotides and the proteins, in the solution having a value of pH being 4 or lower, by flowing a washing solution

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into the separation cell, whereby the cells at ~~the~~ areas except for ~~the~~said one area remain on the ~~area~~substrate and the hybrids remain on ~~the~~said one area, the washing solution is recovered to recover the proteins liberated from the cell;

wherein, after separating the single-stranded polynucleotides not forming the hybrids, whereby the hybrids remain on ~~the~~said one area, by electrophoresis under the DC field applied the surface of ~~the~~said one area, by flowing the washing solution into the separation cell, the washing solution is recovered to recover the single-stranded polynucleotides not forming the hybrid;

wherein, after heating the surface of the substrate at ~~the~~said one area ~~of the areas~~ to denature the hybrids at ~~the~~said one area, so as to liberate the single-stranded target polynucleotides into solution, by flowing the washing solution into the separation cell, the washing solution is recovered to recover the single-stranded target polynucleotides liberated from the cell; and

wherein, by repeatedly changing a position ~~of the~~ to a different area from said one area ~~of the areas~~, the washing solution is recovered to recover, separately, the proteins, the single-stranded polynucleotides not forming the hybrid, and the single-stranded target polynucleotides, for each of the plurality of independent areas.

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31. (Currently Amended) An apparatus according to claim 30, wherein the captured cell is a white blood cell.

32. (Previously Added) An apparatus according to claim 30, wherein the single-stranded target polynucleotide is mRNA.

33. (Currently Amended) An apparatus for recovering a target polynucleotide in a cell comprising:

a substrate being disposed in a separation cell, wherein the sample solution containing cells is supplied on a surface of the substrate, wherein the substrate has ~~and~~ a plurality of independent areas ~~are formed on the its surface of the~~ substrate;

capturing means for capturing each of the cells one by one separately on each of the plurality of independent areas;

means for applying a DC field onto a surface of one area of the plurality of independent areas;

temperature measuring means for measuring a temperature of the surface of the substrate at said one area ~~of the areas~~;

heating or cooling means for heating or cooling the surface of the substrate at thesaid one area ~~of the areas~~;

controlling means for controlling selectively the temperature of the surface of the substrate at thesaid one

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area on the basis of a temperature information obtained by the temperature measuring means, by controlling the heating or cooling means; and

means for identifying the positions of ~~the~~one areas where ~~the~~a cells to be destroyed ~~are~~is present,

wherein the controlling means controls the heating or cooling means so as to heat the surface of the substrate at ~~said one area of the identified positions~~ to a first predetermined temperature to destroy the cell captured at the surface of ~~the~~said one area of the identified positions, to liberate the polynucleotides and the proteins from the cell captured at ~~the~~said one area of the one of the identified positions, and to denature the polynucleotide liberated from the cell so as to obtain a single-stranded polynucleotide, and the controlling means controls the heating or cooling means so as to cool a solution which contains no polynucleotide and has a pH value of 4 or lower and with which the sample solution on the substrate is replaced, to a second predetermined temperature to form hybrids between the single-stranded polynucleotides and the single-stranded oligonucleotide probes, so as to capture~~ing~~ single-stranded target polynucleotides;

wherein, after separating the single-stranded polynucleotides and the proteins, whereby the hybrids remain

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on ~~thesaid one~~ area ~~of the one of the identified positions~~, by electrophoresis under the DC field applied onto the surface of ~~thesaid one~~ area ~~of the one of the identified positions~~, based on a charge difference between the single-stranded target polynucleotides and the proteins, in the solution having a value of pH being 4 or lower, by flowing a washing solution into the separation cell, whereby the cells at ~~the areas~~ except for ~~thesaid one~~ area ~~of the one of the identified positions~~ remain on the ~~area~~ substrate and the hybrids remain on ~~thesaid one~~ area ~~of the one of the identified positions~~, the washing solution is recovered to recover the proteins liberated from the cell;

wherein, after separating the single-stranded polynucleotides not forming the hybrids, whereby the hybrids remain on ~~thesaid one~~ area ~~of the one of the identified positions~~, by electrophoresis under the DC field applied onto the surface of ~~thesaid one~~ area ~~of the one of the identified positions~~, by flowing the washing solution into the separation cell, the washing solution is recovered to recover the single-stranded polynucleotides not forming the hybrid;

wherein, after heating the surface of the substrate at ~~thesaid one area~~ ~~of the identified positions~~ to denature the hybrids at ~~thesaid one~~ area ~~of the one of the identified positions~~, so as to liberate the single-stranded target